Am. J. Trop. Med. Hyg., 86(4), 2012, pp. 642–648
doi:10.4269/ajtmh.2012.11-0326

Efficacy of Mosquito Traps for Collecting Potential West Nile Mosquito Vectors in a Natural Mediterranean Wetland

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Abstract. Surveillance, research, and control of mosquito-borne diseases such as West Nile virus require efficient methods for sampling mosquitoes. We compared the efficacy of BG-Sentinel and Centers for Disease Control and Prevention (CDC)-CO_2 traps in terms of the abundances of host-seeking and blood-fed female mosquitoes and the origin of mosquito bloodmeals. Our results indicate that BG-Sentinel traps that use CO_2 and attractants are as effective as CDC-CO_2 traps for *Culex* mosquito species, *Ochlerotatus caspius*, and they are also highly efficient at capturing *Anopheles atroparvus* host-seeking and blood-fed females with or without CO_2. The CDC-CO_2 trap is the least efficient method for capturing blood-fed females. BG-Sentinel traps with attractants and CO_2 were significantly better at capturing mosquitoes that had fed on mammals than the unbaited BG-Sentinel and CDC-CO_2 traps in the cases of *An. atroparvus* and *Cx. theileri*. These results may help researchers to optimize trapping methods by obtaining greater sample sizes and saving time and money.

INTRODUCTION

In recent years, the Mediterranean Basin has experienced several outbreaks of emerging mosquito-borne viruses such as Chikungunya,[1] dengue,[2] Usutu (USU),[3] and West Nile virus (WNV).[4] In particular, WNV has recently caused outbreaks in the United States and Europe, being the most relevant in Greece, Italy, France, Romania, Portugal, Spain, and Morocco.[5–7] In Spain, the circulation of WNV and other closely related flaviviruses has been reported to have occurred since the 1970s,[8] with high seroprevalence levels in birds[9,10] and horses.[11] Recently, cases of WNV-related illness have been reported in both horses and humans[12] (ProMED-mail, Archive Numbers: 20101119.4203 and 20100925.3478). Additionally, WNV and USU have been detected in the mosquito species *Culex perexiguus* and *Cx. pipiens* in the Doñana Natural Area.[13,14]

Surveillance, research, and control of mosquito-borne diseases all require a good knowledge of mosquito populations and their interactions with the different vertebrate hosts. Sampling host-seeking females—and in particular, disease vector species—plays an important part in understanding mosquito population dynamics, spatial distribution, and arbovirus surveillance.[15–17] However, the sampling of blood-fed females (i.e., engorged) is essential for assessing mosquito blood-feeding patterns, characterizing disease transmission cycles, and identifying key vectors and their hosts, and all these factors play an important role in the epidemiology of vector-borne diseases.[18–20]

The use of an accurate mosquito trapping method is crucial, because several studies have reported significant differences in capture efficiencies between methods.[21,22] Centers for Disease Control and Prevention (CDC) light traps supplemented with CO_2 (CDC-CO_2) are routinely used in surveillance programs in many regions in the world and are the most common sampling method used for adult mosquito collection.[22] In recent years, BG-Sentinel traps (BGS) designed by the BioGents Corporation have been used for collecting *Aedes* (Stegomyia) species such as *Ae. aegypti*, *Ae. albopictus*, and *Ae. polinesiensis*.[23–25] This type of trap can be used with a variety of mosquito attractants (e.g., CO_2, BG-lure, or octenol), thereby making it a versatile tool for mosquito community research and surveillance. Nevertheless, only a few studies have investigated their efficacy regarding the capture of mosquito species other than those species of the genus *Aedes*.[26–28] However, biases related to mosquito diet in the fraction of blood-fed females captured could have major implications for the analyses and interpretation of data originating from mosquito bloodmeals. Indeed, some authors suggest that the synergistic effect of octenol (1-octen-3-ol) and CO_2 significantly increases the capture of mosquitoes that feed on mammals.[29–31] It has been proposed that mosquito feeding behavior (mammal or avian hosts) is the most likely explanation for the differences in catching rates generated by the different combinations of traps and/or attractants.[22,29,32] However, to our knowledge, this hypothesis has never been tested by comparing the origin of vertebrate blood in mosquitoes caught with different trap configurations. Furthermore, little is known regarding the efficacy of different trapping methods for collecting fed mosquitoes[19,33]; likewise, the effects of mosquito feeding behavior on collection method efficiency are also poorly known.

In this paper, we evaluate the efficacy of two types of traps, BGS (with and without two specific mosquito attractants—BG-lure and octenol—and CO_2) and CDC-CO_2, in terms of (1) captures of host-seeking females of different mosquito species, (2) captures of blood-fed female mosquitoes, and (3) origin of mosquito bloodmeals in the Doñana Natural Area (Southwestern Spain, Europe).

MATERIALS AND METHODS

Study area and experimental design. The study was conducted from July 12 to July 16 in the Doñana Natural Area (Southwestern Spain) (Figure 1), one of the most important wetlands in Europe for migratory birds. Three localities were chosen as replicates for our experimental design: the Doñana Palace (Palacio de Doñana), surrounded by freshwater marshes and heathlands; the Jose Antonio Valverde Visitor

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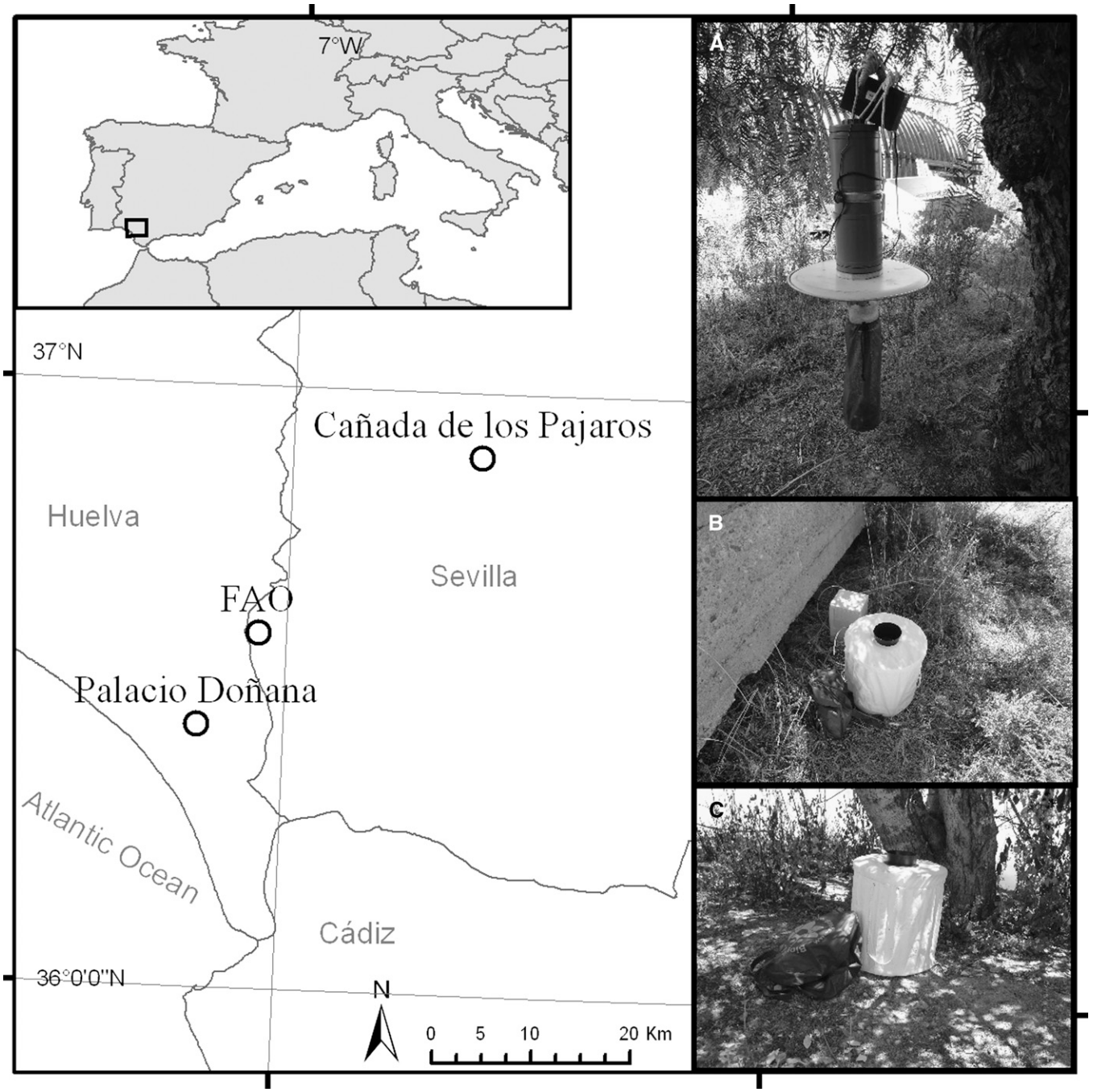

FIGURE 1. Map of the study area and photographs of the different types of traps used: (**A**) CDC with dry ice; (**B**) BGS with dry ice; and (**C**) unbaited BGS without dry ice. This figure appears in color at www.ajtmh.org.

Center (FAO), consisting of wetlands with important breeding colonies of herons and ibis and fields with horses; and the Wildlife Breeding Center of Cañada de los Pajaros, a former gravel pit surrounded by rice fields with a great biodiversity of aquatic bird species (exotic and native) and few mammals. In parallel studies[34] (Roiz D and others, unpublished results), we detected 11 mosquito species, with *Cx. theileri* being the most common followed by *Cx. perexiguus*, *Cx. modestus*, *Cx. pipiens*, and *Anopheles atroparvus*. *Ochlerotatus caspius* is detected flying from the tidal marshes in the coast (mouth of river Gualdalquivir) at 20–30 km apart of the studied localities. Occasionally, we have detected some specimens of *Culiseta longiareolata*, *An. algeriensis*, *Cs. annulata*, *Oc. detritus*, and *Uranotaenia unguiculata*. The experiment was developed in an optimal climate for mosquitoes in the peak period for *Culex* species abundance in the area in crescent moon with an average temperature of 23.8°C (mean minimum of 17.0°C and mean maximum of 31.3°C) and without any rainfall event.

Four trap/attractant configurations were tested: (1) unbaited BGS traps (BioGents, Regensbourg, Germany), (2) BGS traps with CO_2 (generated using dry ice) and BG-Lure® sachets (BioGents, GmbH, Regensbourg, Germany; supplied by AgriSense, Pontypridd, SouthWales, UK), (3) BGS with CO_2 and 1-octen-3-ol (Octenol-Bioquip, Bioquip Products, Rancho

Dominguez, CA) sachets, and (4) CDC mosquito traps with CO_2. The CO_2 used in CDC traps attracts mosquitoes, which are then sucked up with a fan. Octenol is a chemical contained in human breath and sweat, whereas BG-Lure contains a combination of substances found on human skin (lactic acid, ammonia, and fatty acids). BGS traps consist of a white cylindrical container covered with gauze in which ascending currents of the attractant are generated in the center of the trap, where there is a catch bag and a fan that sucks up the mosquitoes. A container with a capacity for around 3 kg dry ice was used with the BGS traps (Figure 1). The CDC-CO_2 traps were hung on low trees, whereas the various different configurations of BGS traps were placed on the ground.

Each of the four different trap configurations were placed at least 200 m from each other in four different sampling points in each of the four localities and thus, generated three sets of a 4×4 Latin square experimental design. To eliminate any position-specific effect, all traps were rotated to the next position every 24 hours four times during the trapping cycle such that each trap at each locality occupied all four positions during the capture period, making a total of 48 traps per nights. Every 24 hours, mosquitoes were collected, transported in dry ice, and stored at –80°C until processed in the laboratory.

Mosquito and bloodmeal identification. Frozen mosquitoes were placed on a piece of white filter paper in a Petri plate on a chill table and identified to species level using appropriate taxonomic keys and a stereo microscope.[35] Specimens belonging to the *Univittatus* complex were identified as *Cx. perexiguus* on the basis of male genitalia.[35] Blood-fed females were identified visually by their dilated red abdomens and stored individually at –80°C until molecular bloodmeal identification could be performed. DNA was isolated from abdominal contents using the HotSHOT protocol as described by Alcaide and others.[34] DNA extracts from the bloodmeals were used as the DNA template in a standard polymerase chain reaction (PCR) assay. PCR products were subsequently used for a nested PCR to amplify a fragment of the vertebrate cytochrome *c* oxidase subunit I (COI) mitochondrial gene using previously described primers (M13BC-FW/BCV-RV1 and M13-FW/BCV-RV2) and thermal cycling conditions.[34] PCR reactions were carried out using a PTC-100 (Programmable Thermal Controller, MJ Research). PCR-amplified products were cleaned up using ExoSAP-IT (GE Healthcare Life Sciences). Sequencing reactions were performed using BigDye 1.1 technology (Applied Biosystems) with BCV-RV2 primer. Labeled DNA fragments were analyzed using an ABI 3130xl automated sequencer (Applied Biosystems). Sequences were checked using Sequencher v.4.5 (Gene Codes Corp.), and COI sequences were assigned to particular vertebrate species using the Barcode of Life Data (BOLD) Systems platform (http://www.boldsystems.org/views/login.php). Positive identifications of host species were based on exact or nearly exact matches (> 98%).

Statistical analysis. The effects of the trapping method on estimates of the relative abundance of host-seeking females, blood-fed females, and origin of bloodmeals (mammal or avian) were analyzed in the five most commonly captured species using generalized linear mixed models (GLMM). GLMM allow dependent variables with error structures that differ from normal distributions (as expected for binary and count data) to be modeled while controlling for independent random variables (in this case, sampling site was nested within locality) to test the statistical significance of a fixed independent variable (trapping method). Negative binomial error distribution and logarithmic link were used for the models; the number of host-seeking or blood-fed females was included as a dependent variable, a procedure that is appropriate for count data. We used a negative binomial rather than Poisson-distributed error to reduce model overdispersion caused by the aggregation of captures.[36] Because we did not capture any *Oc. caspius* with unbaited BGS, we sum one individual to one randomly chosen observation from this category to facilitate model convergence. The presence of mammal blood in bloodmeals was modeled with a binomial distributed error and a logit link in a single model for all mosquito species, with species identity included as a fixed factor. In addition, separate analyses were conducted for the two species that had fed mainly on mammals (*An. atroparvus* and *Cx. theileri*) and those species that had fed mainly on birds (*Cx. modestus*, *Cx. perexiguus*, and *Cx. pipiens*). Least square mean estimates and standard errors of the model were back-transformed before plotting. Statistical analyses were performed in SAS 9.2 with PROC GLIMMIX (SAS-Institute, Cary, NC) fitted by pseudolikelihood.[37]

RESULTS

We collected and identified to species level a total of 33,033 female mosquitoes belonging to 10 species: 15 *An. algeriensis* Theobald, 1903; 4,301 *An. atroparvus* Van Thiel, 1927; 2,454 *Cx. modestus* Ficalbi, 1890; 5,035 *Cx. perexiguus* Theobald, 1903; 219 *Cx. pipiens* Linnaeus, 1758; 20,563 *Cx. theileri* Theobald, 1903; 1 *Cs. longiareolata* Macquart, 1838; 426 *Oc. caspius* Pallas, 1771; 18 *Oc. detritus* Haliday, 1833; and 1 *Ur. unguiculata* Edwards, 1913. In all, 781 of these females were visually identified as blood-fed.

Comparison of traps for capturing host-seeking females. Six mosquito species were captured in sufficient quantity in a representative number of traps to allow for statistical analysis (*An. atroparvus*, *Cx. pipiens*, *Cx. theileri*, *Cx. perexiguus*, *Cx. modestus*, and *Oc. caspius*). In general, the unbaited BGS captured the fewest females (mean ± standard deviation [SD]: 81.5 ± 96) followed by the CDC-CO_2 trap (737.23 ± 669.8), the BG-octenol-CO_2 trap (808.8 ± 642.2), and the BG-lure-CO_2 trap (1,273.7 ± 1,797). *Culex* species and *Oc. caspius* did not show any significant difference between the traps supplied with CO_2 (i.e., CDC and BGS with both attractants) (Figure 2). By contrast, unbaited BGS traps were significantly less efficient than the other three trap configurations. However, for *An. atroparvus*, there were no significant differences between the unbaited BG and the BGS traps with attractants and CO_2; for this *Anopheles* species, the CDC-CO_2 traps caught significantly fewer host-seeking females (Figure 2). The CDC-CO_2 and the BG-lure-CO_2 traps collected the most species ($N = 11$) followed by the BG-octenol-CO_2 trap ($N = 10$). The unbaited BGS traps captured the fewest species ($N = 6$).

Comparison of traps for collecting blood-fed females. The CDC-CO_2 traps captured the fewest blood-fed females of all species (8.7 ± 12.6) followed by the unbaited BGS (15 ± 29.9), the BG-lure-CO_2 (21.3 ± 26.1), and the BG-octenol-CO_2 traps, of which the latter captured almost three times as many species

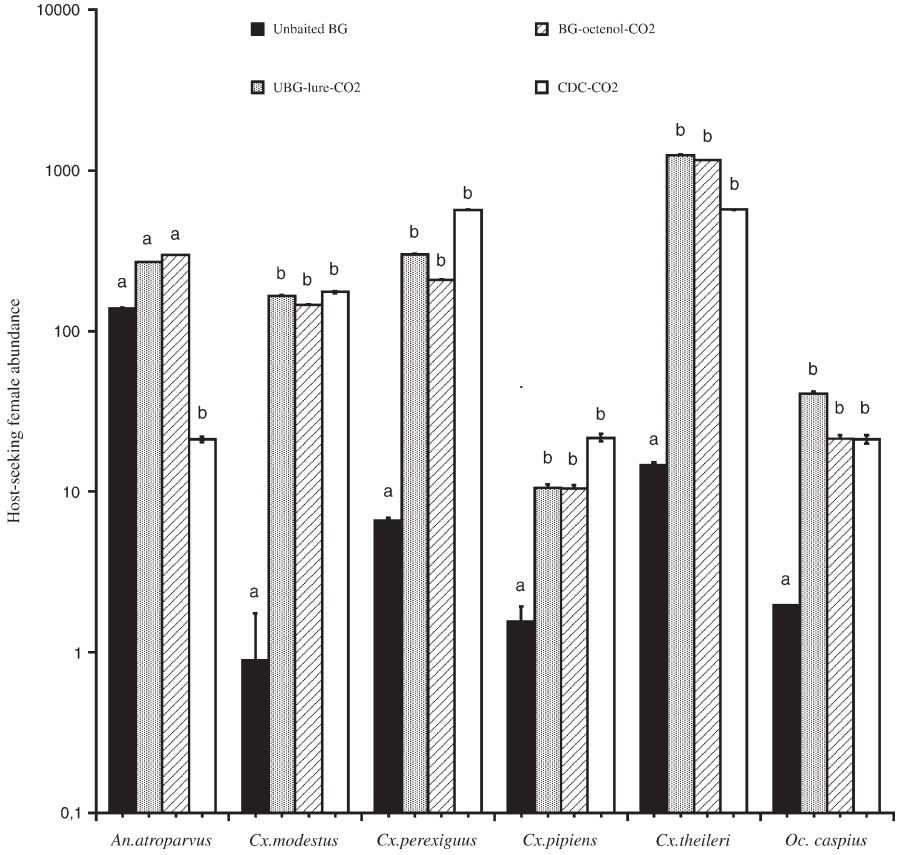

FIGURE 2. Least square means and standard errors of the number of host-seeking females per trap/night. Columns with the same letter are not significantly different ($P < 0.05$).

(23.3 ± 36.1). For the *Culex* species, the unbaited BGS traps captured fewer blood-fed mosquitoes than BGS traps with attractants and CO_2, whereas for *An. atroparvus*, unbaited BGS traps performed as well as BGS with attractants and CO_2 (Figure 3). *Oc. caspius* was not analyzed, because we captured very few blood-fed females.

Host identification and bloodmeal origin. Of the 781 captured blood-fed females, 651 were processed for DNA blood identification. Nevertheless, bloodmeals can be partially digested, and a deterioration of vertebrate DNA occurs (depending on the time elapsed since the mosquito fed[20]). Consequently, only 507 mosquito bloodmeals (77.8%) could be identified to host species level (440 mammals, including 1 human, 65 birds, and 1 reptile). A total of 35 different host species were identified (23 birds, 11 mammals, and 1 reptile). The BG-lure-CO_2 trap was the best for bird bloodmeal trapping (43% host identifications), whereas the BG-octenol-CO_2 trap was the best for mammal bloodmeals (43% host identifications); nevertheless, these results were not statistically significant (data not shown). The GLMM analyses of the origin of the bloodmeals (avian or mammal) in relation to the type of trap used indicate a significant effect for the mosquito species ($F_{4,24} = 7.07$, $P < 0.001$), which means that some species feed more on mammals than others, but no significant effect for the trap used ($F_{3,19} = 2.02$, $P = 0.14$). An analysis of the two mosquito species that fed mainly on mammals, *Cx. theileri* and *An. atroparvus*, reveals a significant effect of the species ($F_{1,11} = 10.93$, $P = 0.007$) and the trap ($F_{3,19} = 4.88$, $P = 0.01$). BGS traps with CO_2 and attractants were significantly better at capturing mammal bloodmeals than the unbaited BGS and CDC-CO_2 traps for both these species (Figure 4). However, we detected no significant differences between traps for captures of ornitophilic species (*Cx. perexiguus*, *Cx. modestus*, and *Cx. pipiens*). Despite some differences (Figure 4), BGS traps with octenol and CO_2 did not attract significantly more mammal-fed mosquitoes than BGS traps with BG-lure and CO_2.

DISCUSSION

Our results show that, for *Culex* species, there were no significant differences in the relative abundance of host-seeking female mosquitoes trapped in CDC-CO_2 and BGS traps with CO_2 and attractants. This finding confirms that the different WNV vector species (*Cx. pipiens*, *Cx. perexiguus*, *Cx. modestus*, and *Cx. theileri*) can be sampled with a similar efficacy using either BGS or CDC-CO_2 traps and that BGS traps are as useful for capturing *Culex* species (WNV vectors) as *Aedes* (Stegomyia) species.[23–25] The same conclusion is valid for *Oc. caspius*. In addition, the effect of the carbon dioxide in both types of CO_2-baited traps was more important for capturing host-seeking mosquitoes than the type of trap itself or the attractants.[21] However, there were no significant differences between the unbaited BG and the BGS traps with attractants and CO_2 for *An. atroparvus* host-seeking females; the CDC-CO_2 traps caught significantly fewer specimens of this mosquito species. These findings, together with data on *An. gambiae*[38] reported by other researchers, could have

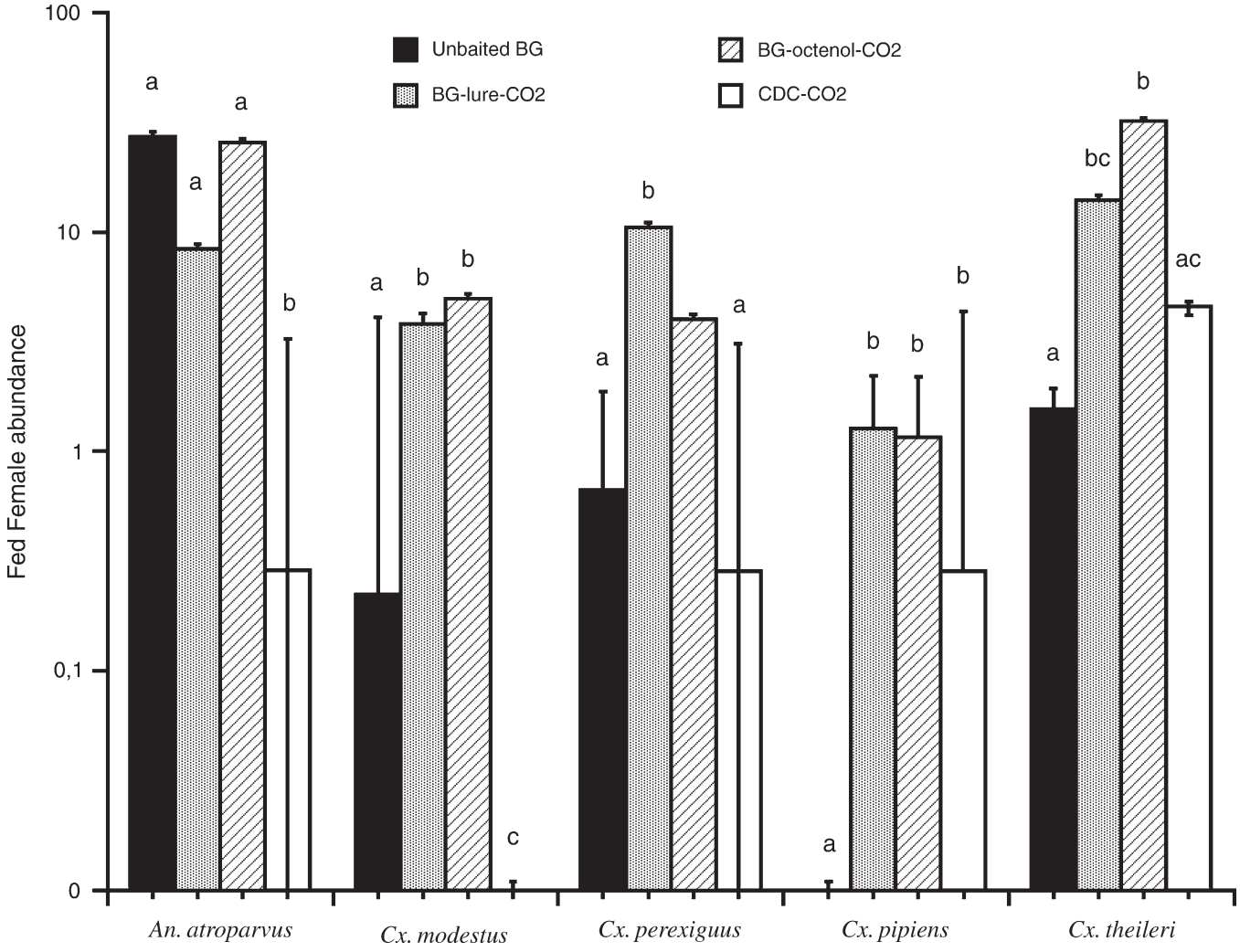

FIGURE 3. Least square means and standard errors of the number of blood-fed females per trap/night. Columns with the same letter are not significantly different (< 0.05).

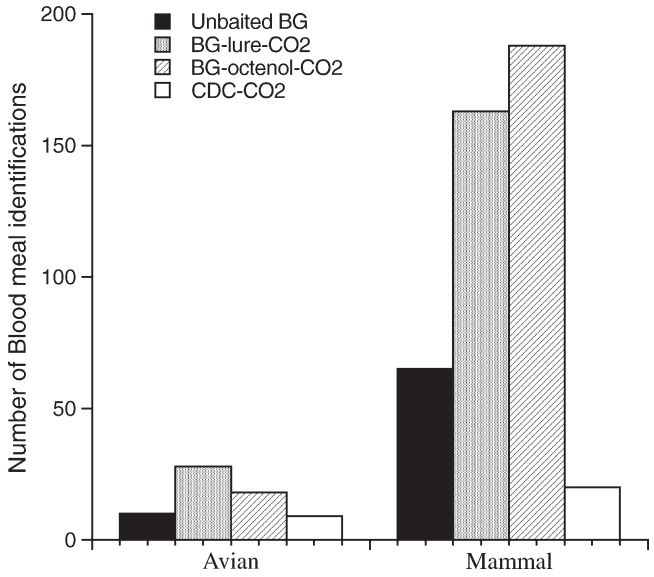

FIGURE 4. Number of mammal or bird meal identifications in the five studied mosquito species.

important implications for the capture of malaria mosquito vectors,[39] especially in areas where access to CO_2 is difficult.

Interestingly, the CDC-CO_2 traps are, in general, the least efficient way of capturing fed females. In fed *Culex* females, BGS traps with attractants and CO_2 are the best trapping method, although for *An. atroparvus*, unbaited BGS traps perform as well as BGS traps with CO_2. Our findings highlight the importance of choosing the type of traps to be used when designing a field study. Given the abundance of mosquitoes and the relative abundance of fed females during our study, the collection of 1,000 blood-fed females using BG traps with CO_2 and an attractant should be completed in 43–47 trap-nights. However, more than the double (115 trap-nights) the number would have been necessary if trapping with CDC-CO_2 traps. In the same way, 10,000 unfed females could be easily trapped using 8 trap-nights with BG-Lure CO_2 traps, but 122 trap-nights would be necessary if trapping with unbaited BG traps.

To our knowledge, this study is the first to combine an analysis of mosquito trapping efficacy with an analysis of the origin of bloodmeals for two commonly used types of traps. All sampling devices used to survey mosquito populations possess different levels of efficacy and potentially target different mosquito species; they are more selective for a specific

fraction of the mosquito community.[22] In fact, it has been proposed that the addition of octenol and/or lactic acid (one of the components of the BG-lure attractant) increases the efficiency of traps with dry ice.[40] Octenol is a common volatile in the emanations of herbivorous mammals[41] and therefore, has been proposed as an attractant for mosquitoes that feed predominately on those vertebrates.[42] Additionally, several researchers state that the combination of octenol and CO_2 increases the collection rates for certain species[31,40,43] but not for others,[40,42,44,45] and therefore, in general, results are not uniform.[22] However, we did not detect any differences in the responses of these mosquito species to BGS traps baited with the combination CO_2-octenol or CO_2–BG-lure. In the Doñana area, our data indicate that *Cx. perexiguus*, *Cx. pipiens*, and *Cx. modestus* are generalist species with a preference for birds (70–80% of bloodmeals); *Cx. theileri* is also generalist but has a preference for mammals (87% of the bloodmeals), whereas *An. atroparvus* is a specialist in mammals (Muñoz J, and others, unpublished results). In fact, BGS traps with CO_2 and attractants performed significantly better in capturing mammal bloodmeals than the unbaited BGS and CDC-CO_2 traps for both mammophilic species (*An. atroparvus* and *Cx. theileri*). These differences could be because of the ability of octenol and lactic acid to simulate mammal hosts and may bias studies of diet comparison.[18,40,46–48] However, we detected no differences among trap configurations in relation to the captures of bird bloodmeals from ornitophilic species, and consequently, no important bias for analyzing bloodmeal diets for those species was detected. Interestingly, we also detected several bird bloodmeals in *An. atroparvus* (12 of 185) from La Cañada; this finding is unusual, because this species is always described as mammophilic,[35] although it is worth remarking that the bloodmeal origin in mosquitoes depends on not only the mosquito species but also, the composition of the vertebrate community. The opportunistic feeding behavior of *Culex* species together with the heterogeneity of host communities have important consequences for the epidemiology of WNV and other arboviruses.[18] Our study contributes new insights that could improve knowledge of zoonotic vector-borne disease patterns through an optimization of trapping tools.

In conclusion, BGS traps with CO_2 are highly suitable for monitoring the *Culex* mosquito species that are vectors of WNV in Mediterranean wetlands and similar habitats, which are priority areas for monitoring virus introduction and amplification.[49] BGS traps are suitable for monitoring *Oc. caspius* and are also highly efficient for capturing *An. atroparvus* with or without CO_2. Comparison with other traps, such as Mosquito Magnet or Zumba traps[50,51] and attractants, with and without CO_2 and development of ornitophilic lures are important keystones to the ultimate objective of improving trapping efficiency for several mosquito-borne diseases such as WNV and USU. Such an evaluation of the efficacy of the different trapping methods and their biases is essential if we are to provide researchers and fieldworkers with accurate tools for targeted trapping (that is, to point to the fraction of the mosquito community that is of greatest ecoepidemiological importance).

Received May 20, 2011. Accepted for publication October 21, 2011.

Acknowledgments: We would like to thank Esmeralda Perez Morueta from the Doñana Biological Station, Seville, Spain, and Juani Moreno from the Mosquito Control Service, Huelva, Spain, for their help and technical support.

Financial support: This work was partially supported by Projects P07-RNM-02511, RNM118, and RNM157 of the Junta de Andalucia and European Commission EuroWestNile FP7 Project 261391.

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